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AN EXPERIENCE USING POSTOPERATIVELY COLLECTED ORTHOPAEDIC NONWASHED FILTERED SHED BLOOD OBTAINED FROM KNEES AND HIPS AS A SOURCE OF AUTOLOGOUS RED BLOOD CELLS

BY

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This study was done to evaluate the in vitro quality of shed blood collected with or without acid-citrate-dextrose (ACD, NIH, Formula A) from the knee and hip within the 12-hour period following orthopaedic surgery. The quality of the 450 to 500 ml of shed blood collected with or without ACD was similar whether collected within 4 hours after surgery, between 4 and 6 hours after surgery, or more than 6 hours after surgery: the hemoglobin concentration was 11.5 gm%, hematocrit 34 V%, WBC count 4,800/mm^3, plasma hemoglobin 250 mg%, fibrinogen level less than 20 mg%, factor V clotting

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protein less than 10% of normal, factor VIII clotting protein 45% of normal, anti-thrombin III level 45% of normal, plasminogen level 55% of normal, protein C level 100% of normal, and fibrin degradation products of 1,000 ug/ml.

A total of 205 units of nonwashed shed blood were reinfused into 153 patients and clinical responses were observed. Hematologic and plasma protein levels were measured in 126 patients who received 170 units of nonwashed shed blood. Measurements were made prior to and 1 hour and 24 hours after the transfusion of 1 to 4 units of shed blood which was filtered but not washed prior to transfusion.

Two of 99 patients (2%) reinfused with shed blood collected within the 6-hour postoperative period exhibited febrile reactions compared with 12 of 54 patients (22%) who had febrile reactions after receiving shed blood collected over a 6- to 12-hour period.

There were no significant differences in hemoglobin concentration or hematocrit value, plasma protein levels or platelet counts in patients who received 1.3 units of autologous nonwashed filtered shed blood whether or not the blood was collected in the ACD anticoagulant. Following reinfusion through a 40 micron Pall screen filter, no clinical bleeding or abnormalities in patient clotting proteins or platelet counts were observed.

ABSTRACT

This study was done to evaluate the in vitro quality of shed blood collected with or without acid-citrate-dextrose (ACD, NIH Formula A) from the knee and hip within the 12-hour period following orthopedic surgery. The quality of the 450 to 500 ml of shed blood collected with or without ACD was similar whether collected within 4 hours after surgery, between 4 and 6 hours after surgery, or more than 6 hours after surgery: the hemoglobin concentration was 11.5 gm%, hematocrit 34 V%, WBC count 4,800/mm^3, plasma hemoglobin 250 mg%, fibrinogen level less than 20 mg%, factor V clotting protein less than 10% of normal, factor VIII clotting protein 45% of normal, anti-thrombin III level 45% of normal, plasminogen level 55% of normal, protein C level 100% of normal, and fibrin degradation products of 1,000 ug/ml.

A total of 205 units of nonwashed shed blood were reinfused into 153 patients and clinical responses were observed. Hematologic and plasma protein levels were measured in 126 patients who received 170 units of nonwashed shed blood. Measurements were made prior to and 1 hour and 24 hours after the transfusion of 1 to 4 units of shed blood which was filtered but not washed prior to transfusion.

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INTRODUCTION

Autologous blood is now being utilized in elective and emergency surgery to avoid the potential of transmission of infectious diseases such as non-A and non-B hepatitis and acquired immune deficiency syndrome (AIDS) associated with homologous blood products. Homologous blood products in the management of orthopaedic patients are now being avoided in favor of pre-deposit autologous blood[7,17,18,35,46], intra-operative autotransfusion[9,19,22-25,28,29,34,36,47,48], hemodilution, and postoperative autotransfusion[12,37,38,41]. Autologous whole blood can be collected and stored in the liquid state at 4 C in CPDA-1 for 35 days and autologous red cells can be stored in additive solutions like ADSOL from Baxter Laboratories, Nutricel from Cutter Laboratories, and Optisol from Terumo, at 4 C for 42 days and preserved in the frozen state for months and years prior to the elective orthopaedic procedures[42,45]. During elective and emergency orthopaedic surgical procedures, intra-operative salvage of autologous blood using heparin and washing of the red cells prior to reinfusion can be utilized[19,24,47,48]. Another alternative for orthopaedic patients is to salvage autologous shed blood following orthopaedic surgery for reinfusion of nonwashed blood and/or washed red blood cells.

This study was done to assess the in vitro quality of shed blood obtained postoperatively following knee and hip surgery. The effects of the ACD anticoagulant (NIH, Formula A) in the collection of post-operative orthopaedic shed blood and of the time period in which the blood

was collected were assessed. In addition, the safety and therapeutic effectiveness of reinfused nonwashed shed blood through a 40 micron screen filter were evaluated.

MATERIALS AND METHODS

One-hundred fifty-three (153) orthopaedic patients, 82 females and 71 males, with an average age of 68 were included in the study. Each patient signed informed consent forms approved by the institutional review board before the study was undertaken.

The procedures included total hip replacements in 86 patients, bilateral total knee replacements in 53 patients, and bilateral total hip replacements in 14 patients. Single total knee replacements were not studied because of the limited amount of postoperative drainage.

Hip replacements were performed through a posterior approach with the patient in the lateral decubitus position. Cemented, uncemented, and hybrid-type techniques were used with titanium femoral stems and polyethylene acetabular liners. Total knee replacements were performed with tourniquet control. The tourniquet was not released until after wound closure. All wounds were prepared with alcohol and iodine impregnated steri-drapes and irrigated with a solution containing 50,000 units per liter of bacitracin. Patients with known malignancy or infection were not reinfused.

Blood evacuation tubes of polyvinylchloride (PVC, 1/8-1/4" diameter) were inserted in a routine fashion into the knee and hip joints during wound closure. The shed blood was collected into a sterile PVC tubing system containing a 240 micron pre-filter into a Solcotrans unit (Solco Basle, Inc., Hingham, MA) with or without the ACD (NIH, Formula A) antiocagulant[11]. In the case of simultaneous bilateral total joint

replacement, the drainage tubes were connected via a Y-connector to a single collection unit. The Solcotrans was used to collect 450 to 500 ml of blood, and when ACD was used, a 40 ml volume was added using a sterile technique. Gravity or a negative pressure of less than 100 mm of mercury was used during collection which took as long as 12 hours. The 450 to 500 ml of shed blood collected with or without ACD was reinfused through a 40 micron Pall screen filter over a 1- to 2-hour period.

Samples of the shed blood were collected into Na_2EDTA anticoagulant and assayed for hemoglobin concentration (Hb), hematocrit value (Hct), red blood cell count (RBC), and white blood cell count (WBC) using the Coulter Counter S-PLUS (Coulter Electronics, Edison, NJ). Samples of the shed blood were collected in special tubes containing thrombin and epsilon aminocaproic acid (EACA), the blood was spun, the supernatant removed, and the supernatant was frozen at -80 C until it was assayed for fibrin degradation products[4]. A sample of shed blood was collected into sodium citrate, the blood centrifuged, and the plasma frozen at -80 C until assayed for: plasma hemoglobin (mg%)[8], factors V and VIII clotting proteins[5,14], anti-thrombin III (%) by chromogenic assay[1], plasminogen by chromogenic assay[15], and protein C (%) by chromogenic assay[33]. Aerobic and anaerobic cultures were done as previously described[45]. Before and after reinfusion of the shed orthopaedic drainage, the vital signs of the patients were monitored, and all reactions were recorded. Patient blood samples were collected prior to reinfusion of 1 to 4 units of shed nonwashed filtered blood. In 86 patients, blood samples were collected

1 hour after the reinfusion of the first unit, in 37 patients samples were collected 1 hour after the second unit, and in 2 patients 1 hour after the third unit. The patient blood samples were collected and assayed in the same manner as the shed blood samples. Platelet counts in the patients' blood were measured using the Coulter S-PLUS (Coulter Electronics, Edison, NJ).

In addition to autologous shed blood transfusions, autologous and homologous liquid preserved whole blood and red blood cell cell concentrates were administered to these patients. The autologous and homologous blood was collected in 450 ml volumes and stored as either whole blood or as red blood cell concentrates with hematocrits of 70V%. When the acid-citrate-dextrose (ACD) anticoagulant or citrate phosphate dextrose (CPD) anticoagulant was used, the units were stored at 4 C for 21 days. When the citrate phosphate dextrose (CPD) anticoagulant supplemented with adenine (CPD-A1) was used, the units were stored at 4 C for 35 days. When blood was collected in the citrate phosphate dextrose anticoagulant, the plasma was removed, and the red blood cell concentrates were stored in an additive solution containing adenine, glucose, mannitol, and sodium chloride (referred to as ADSOL) at 4 C for 42 days. Some of the units were washed prior to transfusion and others were

transfused without washing.

During the post-operative period, one to three units of autologous red blood cells were transfused to each of 54 patients; one to 4 units of homologous red blood cells were transfused to each of 46 patients, and 6 patients received 1 or 2 units of autologous red blood cells together with 1 or 3 units of homologous red blood cells. The autologous and homologous liquid preserved blood units were administered to 106 of 153 patients between the one-hour and 24-hour postoperative period following the reinfusion of the autologous shed blood. The 24 hour measurements reflected the effects of the autologous shed blood reinfusion and the autologous and homologous liquid preserved blood transfusions.

Analyses were performed using the Statistical Analysis System (SAS Institute, Inc., Cary NC) licensed to Boston University. The data are reported as means and standard deviations. A two factor analysis of variance (ANOVA) was performed to test the effects of collection of the blood with or without ACD and from the hip or the knee. A p value of <0.05 was considered significant. Since there were no significant interactions present, the main effects of anticoagulant and site of collection are reported. Non-paired t-tests were used to assess measurements in shed blood collected in less than 4 hours, from 4 to 6 hours, and greater than 6 hours.[39] Paired t-tests were used to

compare the hematologic measurements and plasma protein levels in the patients prior to and 24 hours after the transfusions. An overall p value of ≤ 0.05 was considered significant, however, when to simultaneous comparisons were made, the significance level was adjusted to ≤ 0.025 as described by the Bonferroni method.[32]

RESULTS

The mean collection time for the first unit was 5.6 hours, with a standard deviation of 2.9 hours and a range of 1 to 12 hours. For the second unit the collection time was 6.3 hours, with a standard deviation of 3.0 hours and a range of 2 to 12 hours. Sixty-four per cent of the units were collected and reinfused within 6 hours. The quality of the shed blood collected with and without ACD and reinfused within 4 hours, 4 to 6 hours, and greater than 6 hours was similar, except for minor but significant increases ($p < 0.025$) in white blood cell count for the shed blood collected with ACD during the 4- to 6-hour period and for the shed blood collected without ACD for greater than 12 hours compared to the shed blood collected within 4 hours with and without ACD (Table 1). Clotting was not observed in the shed blood whether or not ACD was used during the collection. The mean hemoglobin concentration was 11.5 g%, hematocrit 34 V%, white blood cell count 4,800/mm^3, plasma hemoglobin level about 250 mg%, fibrinogen level less than 20 mg%, factor V clotting protein of less than 10% of normal, factor VIII clotting protein of 45% of normal, anti-thrombin III level of 45% of normal, plasminogen level of 55% of normal, protein C level of 100% of normal, and fibrin degradation products of 1,000 ug/ml (Table 1).

Samples containing known levels of factor V and factor VIII were used to establish a standard curve for factors V and VIII assays. The factor V level was 80%, with a range of 60-100%, and the factor VIII level was 100%, with a range of 50-150%, for a pool of normal citrated

plasma prepared from ten healthy volunteers.

One hundred-ten (110) units were cultured both aerobically and anaerobically. One unit was positive for Enterococcus. The second unit collected from the same patient had no growth. No reactions were observed following the reinfusion of either unit.

One-hundred-fifty-three (153) patients were reinfused with a total of 205 units of nonwashed filtered shed blood. Data are reported on 126 patients who received a total of 170 units. Eighty-six (86) patients each received 1 unit, 37 each received 2 units, 2 patients received 3 units each, and 1 patient received 4 units. The mean volume of shed blood reinfused per unit was 453 ml, and a mean number of 1.3 units per patient with a range of 1 to 4 units.

Ten percent of the patients exhibited pyrogen reactions with shaking chills and fever following reinfusion of the autologous non-washed filtered shed blood. Of 99 patients who received autologous nonwashed filtered shed blood collected within 6 hours, two febrile transfusion reactions were observed (2%). Of 54 patients who received autologous nonwashed shed blood collected for longer than 6 hours, with an average collection time of 8 hours, 12 febrile transfusion reactions were observed (22%). All reactions resolved rapidly with the administration of anti-pyretic drugs and discontinuation of the transfusion. There were no episodes of hypotension, hemoglobinuria, or coagulopathy.

Comparisons were made of the in vitro quality of the first unit of shed blood collected with and without ACD from the knee or hip of

each patient (Table 2). Shed blood collected from the knee and hip had significantly reduced factor VIII clotting protein and protein C levels when the ACD anticoagulant was used than when the anticoagulant was not used (Table 2). There were significant differences in the clotting proteins and plasma hemoglobin levels between shed blood collected from the knee and that collected from the hip; factor VIII clotting protein and plasminogen levels were significantly increased, protein C was significantly reduced, and the plasma hemoglobin level was significantly greater in the shed blood obtained from the hip compared to that from the knee (Table 2).

The second unit was studied to compare the quality of shed blood collected with and without ACD from the knee and hip (Table 3). The shed blood collected from the knee and hip had significantly lower protein C levels when the ACD anticoagulant was used than when it was not (Table 3). Factor VIII clotting protein was significantly higher and protein C and anti-thrombin III levels significantly lower in shed blood from the hip than in shed blood from the knee (Table 3).

In the two patients from whom three units of shed blood were collected, and the one patient from whom four units of shed blood were collected, the hematocrit of the third unit was 25 V%, hemoglobin concentration was 8.7 g%, and plasma hemoglobin level was 36 mg%. The plasma protein levels in the third unit of shed blood were similar to levels observed in the first and second units.

The effects of the transfusion of a single unit, two units, and three or four units of nonwashed shed blood are reported in Tables 4, 5, and 6. In the patients transfused with one to four units of shed

blood collected with and without ACD, the hemoglobin concentration, hematocrit value, and white blood cell count did not change significantly during the 24-hour posttransfusion period. The platelet count decreased significantly at the 24-hour posttransfusion period but was always greater than 150,000/mm^3. The mean plasma hemoglobin level 1 hour after transfusion of the first and second units was less than 20 mg%, but 24 hours after transfusion was similar to the pre-transfusion level. Fibrinogen and factor VIII levels increased significantly 24 hours after the transfusion, and anti-thrombin III, plasminogen, and protein C levels showed a slight but significant decrease during the 24-hour post-transfusion period. There was no significant change in the other measured parameters 24 hours after transfusion of one or two units of shed blood collected with or without the ACD anticoagulant.

Hematologic and plasma protein measurements in patients transfused with 2, 3, or 4 units of shed nonwashed filtered blood were similar to those in patients who received a single unit of shed blood (Tables 4, 5, and 6).

DISCUSSION

For 20 years there has been debate about whether or not shed blood obtained from the mediastinum should be anticoagulated upon collection and washed prior to reinfusion[13,21,37,38,40]. Washing mediastinal blood reduces the anticoagulant used for collection, the plasma hemoglobin and extracellular potassium levels, and the fibrinogen-fibrin degradation products and D-dimer fragments produced by the clotting and lysis of the shed blood and reduces the products of platelet activation and lysis, i.e., beta thromboglobulin, thromboxane A2, serotonin and lactic dehydrogenase, the products of white blood cell activation and lysis, the products of complement activation, and tissue debris-microaggregates. Shed postoperative orthopaedic blood, in addition to these substances, may contain methyl-methacrylate monomer, local antibiotics, fat, and bone chips.

There have been no reports on the survival of human red blood cells obtained from postoperative orthopaedic drainage. Dog studies have been done to assess the viability and function of dog red blood cells collected intraoperatively from the abdominal cavity and reinfused either as nonwashed filtered blood or as washed filtered red blood cells. After reinfusion of either nonwashed blood or washed red blood cells, 24-hour posttransfusion survivals were 90%, lifespan and oxygen transport function were normal, and hemolysis was minimal[20].

To evaluate the clotting and lysis of blood which occurs during

collection of orthopaedic drainage, we studied baboon blood which has many similarities to human blood[43]. The baboon blood was collected into a plastic bag without any anticoagulant and stored at 22 C for as long as 72 hours[16]. The red blood cells were isolated from the clotted blood and the red blood cells were washed before autotransfusion through a blood filter. The red blood cells that had been stored at 22 C for 24 hours before washing and reinfusion had a 24-hour post-transfusion survival of 90%, normal lifespan, and normal oxygen transport[16].

Autologous human red blood cells salvaged from patients undergoing cardiopulmonary bypass and vascular surgical procedures and washed prior to reinfusion exhibited posttransfusion survival values similar to those of fresh blood[2,3]. The results of these studies by Ansell and associates[2,3], as well as those from our studies in the dog and baboon on intraoperatively and clotted-lysed-washed red blood cells[16,20] showed survival and function values similar to those of liquid-preserved blood stored at 4 C for less than 1 week, and viability and function better than that of liquid-preserved blood stored at 4 C for longer than 1 week. The degree of hemolysis in the shed blood from the knee and hip was about 250 mg%, a value lower than that reported for mediastinal shed blood.

The data indicate that the ACD anticoagulant is not necessary for the collection of shed blood drainage obtained postoperatively from the knees and hips: results were similar for shed blood collected with and without ACD for as long as 12 hours. There was a significantly reduced

level of factor VIII clotting protein observed in the first and second units of shed blood obtained from the knees compared to the hips, and we have no explanation for this. When knee and hip shed blood was collected into the ACD anticoagulant, factor VIII and protein C levels were significantly reduced: the 5.0 pH of the ACD anticoagulant solution may be responsible for the reductions in factor VIII clotting protein and protein C levels. The citrate in the shed blood may adversely affect myocardial function and hemodynamic measurements in hypothermic patients[44], and elimination of the citrate allows for reinfusion of any volume of shed blood.

In this study, febrile transfusion reactions were related to the duration of the shed blood collection. A 2% incidence of febrile reactions was observed when the shed blood was collected within 6 hours or less (i.e., 2/99), and a 22% incidence was observed for shed blood collected for longer than 6 hours (i.e., 12/54). All the febrile reactions resolved rapidly upon administration of anti-pyretic drugs and discontinuation of the transfusion. Bacterial contamination of the shed blood did not occur. Although the etiology of the febrile reactions cannot be stated with certainty, the most likely explanation appears to be the presence of an exogenous pyrogen, the source of which is being investigated. The source of non-hemolytic transfusion reactions following reinfusion of autologous shed blood must be identified and eliminated. A way to reduce this potential problem is to collect and reinfuse the blood drainage from the knees and hips within 6 hours.

No identifiable effects of the fibrinogen-fibrin degradation

products in nonwashed orthopaedic shed blood on platelet function and plasma clotting proteins were found in this study but still remain a concern[6,10,13,26,27,30,40]. The levels of fibrin degradation products (FDP) in the orthopaedic wound drainage were higher than those reported for mediastinal shed blood. This difference was due to the significantly higher level of fibrinogen in the orthopaedic patients than in the hemodiluted patients during cardiopulmonary bypass surgery.

This study also assessed the effects of a mean of 1.3 units of nonwashed filtered shed blood reinfused into adult patients undergoing orthopaedic surgical procedures on the knees and hips. The total volume of nonwashed shed blood reinfused into the patient was 10 to 15% of the patient's blood volume. Two patients received three units of shed blood, and one patient received four units. No untoward effects were observed either clinically or from the laboratory measurements on the patient's clotting proteins and platelets with the small volume of reinfused shed blood. There was no evidence that the fibrinogen-fibrin degradation products produced any coagulopathy by interfering with the function of the platelets or fibrinogen. Further studies are necessary to determine how many units of nonwashed shed blood obtained postoperatively from orthopaedic patients can be reinfused without producing a coagulopathy.

As with all research projects, this study raises as many questions as it answers. No determination of the pathophysiologic effects of the reinfusion of shed nonwashed blood with elevated levels of C3a desArg was made. Moore and associates[31] have reported that patients who

received ventilator support for more than 1 day exhibited plasma C3a desArg levels 2 hours after cardiopulmonary bypass surgery that were nearly twice the levels seen in patients uneventfully weaned from ventilator assistance. Further studies are needed to assess the effects of the fat, local antibiotics, and methyl methacrylate monomer in the shed orthopaedic blood.

Although this study did not answer all questions, it did confirm that the collection and reinfusion of unwashed orthopaedic shed blood without the ACD anticoagulant produced no deleterious effects other than the febrile reactions. To minimize the potential for febrile transfusion reactions, shed blood should be reinfused within 6 hours of collection. Moreover, shed blood should not be collected when the patient is suffering from a known malignancy or prosthetic infection.

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TABLE 1

IN VITRO MEASUREMENTS OF POSTOPERATIVE SHED BLOOD COLLECTED FOR UP TO 12 HOURS WITH AND WITHOUT ACD FROM THE KNEE AND HIP

	TIME OF COLLECTION								
	<4 HOURS			4-6 HOURS			>6 HOURS		
	ACD	NO ACD	COMBINED	ACD	NO ACD	COMBINED	ACD	NO ACD	COMBINED
HEMATOCRIT (V%)									
MEAN:	32	36	34	32	35	34	36	32	34
SD:	8	6	7	9	8	8	12	10	11
N:	13	30	43	9	15	24	12	23	35
RANGE:	16-40	25-48	16-48	20-47	19-46	19-47	15-55	16-58	15-58
HEMOGLOBIN (g/dl)									
MEAN:	10.8	12.3	11.9	10.9	11.5	11.3	12.4	11.1	11.5
SD:	2.7	0.4	2.5	3.2	2.6	2.8	4.2	3.4	3.7
N:	13	30	43	9	15	24	12	23	35
RANGE:	5.4-14.6	8.8-17.1	5.4-17.1	6.9-16.2	6.4-15.7	6.4-11.2	4.9-19.1	5.5-19.7	4.9-19.7
RED BLOOD CELL COUNT ($X10^6/mm^3$)									
MEAN:	3.38	3.74	3.60	3.76	3.88	3.81	4.25	3.47	3.76
SD:	0.77	0.89	0.86	0.91	0.92	0.90	1.17	1.07	1.15
N:	17	30	47	9	15	24	13	22	35
RANGE:	2.02-4.42	1.00-5.21	1.00-5.21	2.21-5.13	1.99-5.49	1.99-5.49	1.55-6.23	1.73-5.28	1.55-6.23
WHITE BLOOD CELL COUNT ($X10^3/mm^3$)									
MEAN:	3.4	4.6	4.2	5.0*	4.7	4.8	4.3	6.0*	5.3
SD:	1.7	0.4	1.9	1.4	2.2	1.9	2.1	2.3	2.4
N:	17	30	47	9	15	24	13	22	35
RANGE:	1.9-8.1	1.9-11.4	1.9-11.4	2.8-7.2	1.1-9.1	1.1-9.1	1.0-8.1	2.5-11.0	1.0-11.0
PLASMA HEMOGLOBIN (mg%)									
MEAN:	136	127	192	137	161	152	65	238	203
SD:	123	71	86	69	79	74	45	62	233
N:	7	18	25	6	9	15	4	16	20
RANGE:	13-384	46-285	13-384	49-161	50-298	31-298	7-118	24-936	9-936
FIBRINOGEN (mg/dl)									
MEAN:	<20	<20	<20	<20	<20	<20	<20	<20	<20
SD:	0	0	0	0	0	0	0	0	0
N:	13	27	40	9	14	23	12	23	35
RANGE:	--	--	--	--	--	--	--	--	--

*Significant difference ($p < 0.025$) compared to <4 hour time of collection

TABLE 1 (CONT.)

	TIME OF COLLECTION								
	< 4 HOURS			4-6 HOURS			> 6 HOURS		
	ACD	NO ACD	COMBINED	ACD	NO ACD	COMBINED	ACD	NO ACD	COMBIN
FACTOR VIII CLOTTING PROTEIN (%)									
MEAN:	36	50	45	49	54	52	31	52	44
SD:	22	18	20	41	24	31	14	24	23
N:	13	27	40	9	14	23	12	23	35
RANGE:	10-84	25-85	10-85	17-143	19-105	17-143	10-50	10-112	10-112
FACTOR V CLOTTING PROTEIN (%)									
MEAN:	<10	<10	<10	<10	<10	<10	<10	<10	< 10
SD:	0	0	0	0	0	0	0	0	0
N:	13	27	40	9	14	23	12	23	35
RANGE:	--	--	--	--	--	--	--	--	--
ANTI-THROMBIN III (%)									
MEAN:	45	51	34	56	44	48	54	45	48
SD:	13	16	15	15	16	16	25	10	17
N:	11	29	40	7	15	22	11	21	32
RANGE:	22-61	31-96	22-96	37-74	6-73	6-74	6-106	26-76	6-106
PLASMINOGEN (%)									
MEAN:	54	57	55	50	57	55	48	57	54
SD:	18	16	17	15	15	15	17	15	16
N:	13	29	42	8	15	23	12	21	33
RANGE:	25-91	37-91	25-91	29-77	29-81	29-81	25-80	34-88	25-88
PROTEIN C (%)									
MEAN:	111	121	118	83	110	99	79	116	10
SD:	56	32	42	39	35	38	40	42	4
N:	15	30	45	10	15	25	13	23	3
RANGE:	52-196	75-192	52-196	49-161	38-180	38-180	7-110	56-209	20
FIBRIN DEGRADATION PRODUCTS (ug/ml)									
MEAN:	891	1019	977	728	1088	953	771	930	87
SD:	359	374	370	350	338	379	434	349	23
N:	14	28	42	9	15	24	11	21	3
RANGE:	320-1280	80-1280	80-1280	160-1280	320-1280	160-1280	160-1280	320-1280	16-128

TABLE 2

IN VITRO MEASUREMENTS OF THE FIRST UNIT OF POSTOPERATIVE SHED BLOOD COLLECTED FOR UP TO 12 HOURS WITH AND WITHOUT ACD FROM THE KNEE AND HIP

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE		UNIT COLLECTED INTO NO ANTICOAGULANT		ANOVA p VALUE	
	HIP	KNEE	HIP	KNEE	ACD VS NO ACD	HIP VS KNEE
HEMATOCRIT (V%)						
MEAN:	33	36	34	34		
SD:	11	8	9	7		
N:	27	18	45	24	NS	NS
RANGE:	15-66	20-55	18-58	16-44		
HEMOGLOBIN (g/dl)						
MEAN:	11.3	12.3	11.7	11.8		
SD:	4.0	2.9	3.0	2.3		
N:	27	18	45	24	NS	NS
RANGE:	4.9-22.7	6.9-16.5	6.3-19.7	5.5-15.0		
RED BLOOD CELL COUNT ($X10^6/mm^3$)						
MEAN:	3.59	3.96	3.61	3.79		
SD:	1.02	0.93	1.02	0.80		
N:	22	15	48	23	NS	NS
RANGE:	1.55-5.32	2.12-6.23	1.00-5.49	1.73-5.21		
WHITE BLOOD CELL COUNT ($X10^3/mm^3$)						
MEAN:	4.7	3.3	5.3	4.1		
SD:	1.8	1.3	2.4	1.4		
N:	20	14	48	23	NS	NS
RANGE:	2.3-8.1	1.0-7.2	1.1-11.4	1.9-6.7		
PLASMA HEMOGLOBIN (mg%)						
MEAN:	146	116	220	78		
SD:	59	134	179	66		
N:	13	12	30	14	NS	<0.05
RANGE:	9-239	13-409	24-936	26-298		
FIBRINOGEN (mg/dl)						
MEAN:	35	<20	<20	<20		
SD:	21	0	0	0		
N:	15	13	44	18	NS	NS
RANGE:	20-85	--	--	--		

TABLE 2 (CONT.)

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE		UNIT COLLECTED INTO NO ANTICOAGULANT		ANOVA p VALUE	
	HIP	KNEE	HIP	KNEE	ACD VS NO ACD	HIP VS KNEE
FACTOR VIII CLOTTING PROTEIN (%)						
MEAN:	50	28	60	35		
SD:	27	14	20	11		
N:	27	17	45	22	<0.05	<0.001
RANGE:	10-143	10-67	31-112	10-51		
FACTOR V CLOTTING PROTEIN (%)						
MEAN:	<10	<10	<10	<10		
SD:	0	0	0	0		
N:	12	15	48	23	NS	NS
RANGE:	--	--	--	--		
ANTI-THROMBIN III (%)						
MEAN:	50	54	48	45		
SD:	20	10	16	12		
N:	24	16	44	22	NS	NS
RANGE:	6-106	34-70	26-72	31-61		
PLASMINOGEN (%)						
MEAN:	54	50	60	49		
SD:	16	18	16	12		
N:	26	18	46	22	NS	<0.01
RANGE:	25-83	25-91	34-91	29-74		
PROTEIN C (%)						
MEAN:	95	120	100	152		
SD:	49	62	25	32		
N:	26	22	47	22	<0.05	<0.001
RANGE:	13-146	7-236	38-180	108-209		
FIBRIN DEGRADATION PRODUCTS (ug/ml)						
MEAN:	747	1031	1025	932		
SD:	384	321	349	397		
N:	27	18	44	21	NS	NS
RANGE:	160-1280	640-1280	320-1280	80-1280		

TABLE 3

IN VITRO MEASUREMENTS OF THE SECOND UNIT OF POSTOPERATIVE SHED BLOOD COLLECTED FOR UP TO 12 HOURS WITH AND WITHOUT ACD FROM THE KNEE AND HIP

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE		UNIT COLLECTED INTO NO ANTICOAGULANT		ANOVA p VALUE	
	HIP	KNEE	HIP	KNEE	ACD VS NO ACD	HIP VS KNEE
HEMATOCRIT (V%)						
MEAN:	32	34	34	32		
SD:	11	5	8	10		
N:	3	7	10	10	NS	NS
RANGE:	21-43	25-29	18-58	15-48		
HEMOGLOBIN (g/dl)						
MEAN:	10.6	11.3	11.8	9.7		
SD:	3.5	1.8	3.6	3.7		
N:	3	7	10	10	NS	NS
RANGE:	7.4-14.2	8.5-13.5	6.3-19.7	3.1-15.8		
RED BLOOD CELL COUNT ($X10^6/mm^3$)						
MEAN:	3.55	3.27	3.79	3.42		
SD:	1.11	0.48	0.98	1.00		
N:	4	6	12	12	NS	NS
RANGE:	2.33-5.02	2.64-4.32	2.59-5.96	1.66-5.12		
WHITE BLOOD CELL COUNT ($X10^3/mm^3$)						
MEAN:	5.8	5.9	6.5	6.1		
SD:	3.4	3.8	3.5	2.2		
N:	4	8	13	12	NS	NS
RANGE:	3.9-11.0	2.9-11.9	1.2-14.0	3.1-9.1		
PLASMA HEMOGLOBIN (mg%)						
MEAN:	33	39	67	39		
SD:	33	40	21	9		
N:	3	6	5	3	NS	NS
RANGE:	13-71	12-118	40-87	24-41		
FIBRINOGEN (mg/dl)						
MEAN:	32	46	26	49		
SD:	14	51	24	53		
N:	6	5	16	10	NS	NS
RANGE:	20-46	20-132	20-195	20-160		

TABLE 3 (CONT.)

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE		UNIT COLLECTED INTO NO ANTICOAGULANT		ANOVA p VALUE	
	HIP	KNEE	HIP	KNEE	ACD VS NO ACD	HIP VS KNEE
FACTOR VIII CLOTTING PROTEIN (%)						
MEAN:	42	20	42	29		
SD:	16	12	16	7		
N:	5	8	12	11	NS	<0.001
RANGE:	20-72	8-38	28-84	16-38		
FACTOR V CLOTTING PROTEIN (%)						
MEAN:	<10	13	<10	<10		
SD:	0	8	0	0		
N:	3	5	17	12	NS	NS
RANGE:	--	10-27	--	--		
ANTI-THROMBIN III (%)						
MEAN:	43	66	45	45		
SD:	11	17	8	10		
N:	5	7	12	11	NS	<0.01
RANGE:	26-56	43-96	35-56	36-67		
PLASMINOGEN (%)						
MEAN:	58	61	70	50		
SD:	16	21	14	15		
N:	6	8	12	11	NS	NS
RANGE:	25-77	45-110	45-93	41-84		
PROTEIN C (%)						
MEAN:	58	93	84	114		
SD:	16	34	20	31		
N:	6	8	12	11	<0.05	<0.001
RANGE:	25-77	45-150	53-116	79-177		
FIBRIN DEGRADATION PRODUCTS (ug/ml)						
MEAN:	640	846	873	1120		
SD:	---	327	349	296		
N:	1	7	11	8	NS	NS
RANGE:	---	160-1280	320-1280	640-1280		

TABLE 4

HEMATOLOGIC AND PLASMA PROTEIN LEVELS IN PATIENTS PRIOR TO, AND 1 AND 24 HOURS FOLLOWING THE TRANSFUSION OF ONE UNIT OF NONWASHED SHED BLOOD OBTAINED FROM THE KNEE AND HIP COLLECTED FOR UP TO 12 HOURS WITH AND WITHOUT ACD

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE			UNIT COLLECTED INTO NO ANTICOAGULANT			PAIRED T-TEST p VALUE PRE VS 24-HOUR POSTTRANSFUSION	
		POSTTRANSFUSION			POSTTRANSFUSION			
	PRE	1 HOUR	24 HOUR	PRE	1 HOUR	24 HOUR	ACD	NO ACD
HEMATOCRIT (V%)								
MEAN:	35	33	35	35	32	34		
SD:	4	4	4	4	4	4		
N:	33	22	12	40	43	37	NS	NS
RANGE:	26-43	26-40	29-41	26-44	23-41	23-43		
HEMOGLOBIN (g/dl)								
MEAN:	12.1	11.2	11.8	12.2	11.2	11.7		
SD:	1.4	1.5	1.2	2.6	1.5	1.5		
N:	33	22	12	40	42	37	NS	NS
RANGE:	9.4-15.1	9.1-13.8	10.0-13.8	8.3-25.4	7.9-14.2	7.8-14.8		
PLATELET COUNT ($X10^3/mm^3$)								
MEAN:	268	219	203	286	238	222		
SD:	76	47	38	98	72	91		
N:	33	22	13	40	43	37	<0.01	<0.001
RANGE:	122-409	148-324	162-290	117-577	102-405	58-577		
RED BLOOD CELL COUNT ($X10^6/mm^3$)								
MEAN:	3.84	3.64	3.82	3.88	3.53	3.70		
SD:	0.46	0.45	0.41	0.44	0.50	0.50		
N:	22	21	12	43	47	41	NS	NS
RANGE:	3.03-4.90	2.99-4.42	3.17-4.58	2.92-4.79	2.25-4.43	2.50-4.60		
WHITE BLOOD CELL COUNT ($X10^3/mm^3$)								
MEAN:	8.7	12.8	9.7	10.4	13.9	11.2		
SD:	3.9	4.3	2.4	4.6	5.0	3.9		
N:	23	21	12	43	47	41	NS	NS
RANGE:	4.4-15.1	7.8-23.8	6.1-15.1	3.1-23.7	3.4-25.1	4.8-24.0		
PLASMA HEMOGLOBIN (mg%)								
MEAN:	6.4	10.8	2.0	3.0	12.9	6.8		
SD:	7.6	11.6	0.7	2.4	13.4	9.1		
N:	17	15	5	8	9	8	NS	NS
RANGE:	1-26	1-49	1-3	1-7	2-34	1-27		

TABLE 4 (CONT.)

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE			UNIT COLLECTED INTO NO ANTICOAGULANT			PAIRED T-TEST p VALUE PRE VS 24-HOUR POSTTRANSFUSION	
		POSTTRANSFUSION			POSTTRANSFUSION			
	PRE	1 HOUR	24 HOUR	PRE	1 HOUR	24-HOUR	ACD	NO ACD
FIBRINOGEN (mg/dl)								
MEAN:	286	247	466	266	269	459		
SD:	72	61	114	77	63	148		
N:	27	24	9	22	24	19	<0.01	<0.001
RANGE:	185-550	145-385	300-660	110-520	175-450	270-780		
FACTOR VIII CLOTTING PROTEIN (%)								
MEAN:	113	121	145	147	138	158		
SD:	42	48	54	63	44	56		
N:	27	23	10	20	25	20	<0.05	NS
RANGE:	64-214	59-270	74-260	47-280	53-226	66-323		
FACTOR V CLOTTING PROTEIN (%)								
MEAN:	61	58	63	72	65	74		
SD:	15	14	23	21	17	27		
N:	26	23	9	21	23	16	NS	NS
RANGE:	33-87	38-94	36-116	36-102	31-88	35-145		
ANTI-THROMBIN III (%)								
MEAN:	112	103	84	91	86	76		
SD:	22	21	30	10	9	11		
N:	25	20	5	20	17	17	NS	<0.01
RANGE:	73-183	69-150	56-118	76-111	71-97	56-94		
PLASMINOGEN (%)								
MEAN:	86	99	79	77	88	70		
SD:	13	17	19	13	13	17		
N:	27	23	6	19	18	17	NS	NS
RANGE:	59-112	70-125	55-98	54-108	57-112	44-101		
PROTEIN C (%)								
MEAN:	111	85	76	94	89	71		
SD:	39	21	17	16	14	17		
N:	26	16	12	23	20	17	NS	<0.001
RANGE:	60-198	59-125	49-108	65-141	64-117	45-96		
FIBRIN DEGRADATION PRODUCTS (ug/ml)								
MEAN:	3	71	23	2	90	22		
SD:	7	47	45	6	68	46		
N:	27	24	12	33	31	25	NS	NS
RANGE:	0-20	20-160	0-160	0-20	0-320	0-160		

TABLE 5

HEMATOLOGIC AND PLASMA PROTEIN LEVELS IN PATIENTS PRIOR TO, AND 1 AND 24 HOURS FOLLOWING THE TRANSFUSION OF TWO UNITS OF NONWASHED SHED BLOOD OBTAINED FROM THE KNEE AND HIP COLLECTED FOR UP TO 12 HOURS WITH AND WITHOUT ACD

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE				UNIT COLLECTED INTO NO ANTICOAGULANT				PAIRED T-TEST p VALUE PRE VS 24-HOUR POSTTRANSFUSION	
		POSTTRANSFUSION				POSTTRANSFUSION				
		UNIT 1	UNIT 2			UNIT 1	UNIT 2			
	PRE	1 HOUR	1 HOUR	24 HOUR	PRE	1 HOUR	1 HOUR	24 HOUR	ACD	NO ACD
HEMATOCRIT (V%)										
MEAN:	34	33	33	35	35	32	31	34		
SD:	4	4	3	4	4	3	8	4		
N:	14	15	10	13	21	22	23	16	NS	NS
RANGE:	28-41	28-41	27-37	30-44	22-40	27-38	22-43	27-38		
HEMOGLOBIN (g/dl)										
MEAN:	11.8	11.1	11.0	11.9	11.9	11.1	11.1	11.4		
SD:	1.5	1.3	1.4	1.5	1.5	1.0	1.3	1.2		
N:	14	15	10	13	21	22	23	16	NS	NS
RANGE:	9.2-13.7	9.5-14.2	9.7-13.0	10.0-14.9	7.6-14.3	8.7-12.7	7.6-14.0	9.6-13.1		
PLATELET COUNT ($X10^3/mm^3$)										
MEAN:	281	227	227	207	249	210	200	184		
SD:	63	61	67	38	86	48	56	47		
N:	14	15	10	13	21	21	23	15	<0.001	<0.01
RANGE:	136-371	137-357	156-351	143-280	70-369	111-293	64-276	58-247		
RED BLOOD CELL COUNT ($X10^6/mm^3$)										
MEAN:	3.82	3.72	3.69	3.91	3.80	3.52	3.55	3.58		
SD:	0.32	0.41	0.35	0.52	0.47	0.34	0.46	0.54		
N:	14	15	12	14	21	22	22	15	NS	NS
RANGE:	3.22-4.49	2.97-4.75	3.16-4.17	3.17-5.03	2.32-4.45	2.84-4.11	2.41-4.06	2.50-4.79		
WHITE BLOOD CELL COUNT ($X10^3/mm^3$)										
MEAN:	9.7	11.9	10.9	10.9	11.0	14.0	11.4	12.0		
SD:	5.0	4.9	3.3	3.8	6.9	3.3	3.7	2.9		
N:	14	15	12	14	21	22	22	15	NS	NS
RANGE:	4.1-22.8	3.6-21.9	7.7-17.2	4.1-16.5	2.7-32.2	9.2-22.9	5.0-21.0	6.7-16.0		
PLASMA HEMOGLOBIN (mg%)										
MEAN:	6.6	18.3	2.0	4.0	7.0	16.0	14.0	3.0		
SD:	2.2	22.1	1.0	7.0	7.0	11.0	16.0	3.0		
N:	5	6	3	4	8	11	8	9	NS	NS
RANGE:	3-9	2-50	2-3	1-14	1-17	3-37	1-21	1-11		

TABLE 5 (CONT.)

	UNIT COLLECTED INTO ACID-CITRATE-DEXTROSE				UNIT COLLECTED INTO NO ANTICOAGULANT				PAIRED T-TEST p VALUE PRE VS 24-HOUR POSTTRANSFUSION	
		POSTTRANSFUSION				POSTTRANSFUSION				
		UNIT 1	UNIT 2			UNIT 1	UNIT 2			
	PRE	1 HOUR	1 HOUR	24 HOUR	PRE	1 HOUR	1 HOUR	24 HOUR	ACD	NO ACD
FIBRINOGEN (mg/dl)										
MEAN:	293	245	316	481	272	223	250	431		
SD:	91	48	111	177	100	65	65	110		
N:	15	15	8	12	10	14	6	9	<0.05	<0.01
RANGE:	130-500	150-320	225-540	180-710	180-510	125-320	190-370	275-650		
FACTOR VIII CLOTTING PROTEIN (%)										
MEAN:	135	118	128	137	133	159	119	167		
SD:	72	40	56	45	52	53	36	70		
N:	14	15	6	12	11	16	5	10	NS	NS
RANGE:	49-325	50-218	90-235	65-196	66-214	63-262	75-157	62-264		
FACTOR V CLOTTING PROTEIN (%)										
MEAN:	64	52	52	61	74	64	52	74		
SD:	18	13	9	17	17	14	10	22		
N:	12	15	7	12	11	14	5	8	NS	NS
RANGE:	37-92	32-89	43-66	34-96	51-104	51-93	46-65	48-110		
ANTI-THROMBIN III (%)										
MEAN:	111	106	92	94	92	91	83	78		
SD:	17	17	23	10	8	34	13	11		
N:	12	12	2	6	8	10	5	4	NS	NS
RANGE:	78-140	83-133	76-108	83-109	84-109	37-174	62-95	70-95		
PLASMINOGEN (%)										
MEAN:	87	90	74	68	70	82	98	60		
SD:	13	13	7	10	16	10	17	19		
N:	14	14	3	8	8	10	5	4	<0.005	NS
RANGE:	60-109	62-109	67-80	50-78	55-107	64-99	81-123	38-80		
PROTEIN C (%)										
MEAN:	99	90	85	65	98	87	77	67		
SD:	25	20	8	17	18	24	18	16		
N:	13	14	5	10	8	10	5	4	<0.01	NS
RANGE:	69-151	63-128	71-90	20-80	74-124	46-117	51-95	53-88		
FIBRIN DEGRADATION PRODUCTS (ug/ml)										
MEAN:	14	99	95	7	11	103	115	18		
SD:	23	54	10	16	16	78	83	18		
N:	14	15	8	12	11	15	11	10	NS	NS
RANGE:	0-80	40-160	20-320	0-40	0-40	20-320	20-320	0-40		

TABLE 6

HEMATOLOGIC AND PLASMA PROTEIN LEVELS IN PATIENTS PRIOR TO, and 1 AND 24 HOURS FOLLOWING THE TRANSFUSION OF THREE TO FOUR UNITS OF NONWASHED SHED BLOOD OBTAINED FROM THE KNEE AND HIP COLLECTED FOR UP TO 12 HOURS WITHOUT ACD ANTICOAGULANT

UNIT COLLECTED INTO NO ANTICOAGULANT

		POSTTRANSFUSION			
	PRE	UNIT 1 1 HOUR	UNIT 2 1 HOUR	UNIT 3 1 HOUR	24 HOUR
HEMATOCRIT (V%)					
MEAN:	36	33	31	32	33
SD:	3	4	4	3	4
N:	3	2	3	3	2
RANGE:	33-39	30-35	27-34	30-35	30-36
HEMOGLOBIN (g/dl)					
MEAN:	12.4	11.1	10.4	11.0	11.4
SD:	1.0	0.8	1.0	0.7	1.6
N:	3	2	3	3	3
RANGE:	11.4-13.3	10.5-11.7	9.4-11.3	10.3-11.6	10.2-12.5
PLATELET COUNT ($X10^3/mm^3$)					
MEAN:	227	201	156	157	157
SD:	108	71	43	33	21
N:	3	2	3	3	2
RANGE:	133-345	150-251	118-202	114-162	142-171
RED BLOOD CELL COUNT ($X10^6/mm^3$)					
MEAN:	4.02	3.63	3.43	3.61	3.67
SD:	0.25	0.67	0.48	0.40	0.13
N:	3	2	3	3	3
RANGE:	3.77-4.27	3.16-4.09	3.11-3.98	3.34-4.07	3.55-3.80
WHITE BLOOD CELL COUNT ($X10^3/mm^3$)					
MEAN:	9.0	13.6	10.2	10.8	9.6
SD:	1.8	2.5	5.0	4.1	2.9
N:	3	2	3	3	3
RANGE:	7.0-10.5	11.8-15.4	4.6-14.1	6.3-14.1	7.1-12.7
PLASMA HEMOGLOBIN (mg%)					
MEAN:	5	36	6	1	7
SD:	--	--	1	--	8
N:	1	1	2	1	2
RANGE:			5-7		1-12

TABLE 6 (CONT.)

UNIT COLLECTED INTO NO ANTICOAGULANT

		POSTTRANSFUSION			
	PRE	UNIT 1 1 HOUR	UNIT 2 1 HOUR	UNIT 3 1 HOUR	24 HOUR
FIBRINOGEN (mg/dl)					
MEAN:	245	236	202	243	453
SD:	163	125	99	132	221
N:	2	3	3	3	3
RANGE:	130-360	92-320	115-310	135-390	220-660
FACTOR VIII CLOTTING PROTEIN (%)					
MEAN:	125	119	147	106	169
SD:	52	74	38	34	59
N:	2	3	3	3	3
RANGE:	88-161	36-180	105-180	85-145	123-236
FACTOR V CLOTTING PROTEIN (%)					
MEAN:	57	41	51	44	55
SD:	29	30	23	20	21
N:	2	3	3	3	3
RANGE:	36-77	10-69	31-76	26-66	32-74
ANTI-THROMBIN III (%)					
MEAN:	77	61	69	77	86
SD:	7	26	16	7	--
N:	2	2	2	2	1
RANGE:	73-81	42-79	57-80	72-81	--
PLASMINOGEN (%)					
MEAN:	80	96	87	92	68
SD:	13	21	36	30	--
N:	2	2	2	2	1
RANGE:	70-89	81-110	61-112	71-113	--
PROTEIN C (%)					
MEAN:	63	79	59	63	90
SD:	18	44	15	24	--
N:	2	2	2	2	1
RANGE:	50-76	48-110	48-69	46-80	--
FIBRIN DEGRADATION PRODUCTS (ug/ml)					
MEAN:	40	240	160	40	20
SD:	--	113	---	---	0
N:	1	2	1	1	2
RANGE:	--	160-320	---	---	--